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Unexpected stereoselective exchange of straight-chain fatty acyl-CoA α -protons by human α -methylacyl-CoA racemase 1A (P504S)

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α -Methylacyl-CoA racemase (AMACR; P504S) enzyme levels are increased ~10-fold in prostate and other cancers. The enzyme catalyses chiral inversion of 2-methylacyl-CoA substrates by removal of the C-2 proton followed by non-stereoselective reprotonation resulting in a ~1:1 epimeric mixture of 2S- and 2R-methyl products. AMACR is localized in peroxisomes and mitochondria. Both organelles contain straight-chain acyl-CoA esters, which are potential substrates for AMACR.

The present study investigates whether straight-chain acyl-CoA esters are substrates for the enzyme. Incubation of decanoyl-CoA with AMACR in the presence of $^2\text{H}_2\text{O}$ resulted in proton exchange, as judged by ^1H -NMR. The reaction was reversible, as shown by deuterium loss from 2,2'-[$^2\text{H}_2$]-decanoyl-CoA when incubated with AMACR in $^1\text{H}_2\text{O}$ -containing buffer. Incubation of 2-[$^{13}\text{C}_1$]-decanoyl-CoA with AMACR in the presence of $^2\text{H}_2\text{O}$ showed only one proton was exchanged, as judged by the conversion of the substrate ^{13}C -singlet to a triplet in the NMR spectrum. Unexpectedly, chiral derivatization of incubation products followed by ^1H NMR analysis showed that the 2S-proton was exchanged much more frequently than the 2R-proton. Steady-state kinetic analysis of decanoyl-CoA and S-2-methyldecanoyl-CoA esters showed that straight-chain acyl-CoA esters are poor substrates for AMACR, as judged by $k_{\text{cat}}/K_{\text{m}}$ values (114 vs. 37 $\text{M}^{-1} \text{s}^{-1}$).

Straight-chain acyl-CoA esters are AMACR substrates *in vitro*, but these results suggest that AMACR does not substantially catalyse proton-exchange of straight-chain acyl-CoA substrates *in vivo*.

219 words

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